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The Presence of *Helicobacter Pylori* in Cases of Chronic Tonsillitis in Medan

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Abstract. *Helicobacter pylori* is a well known pathogen which is recognized as the most frequent infection of individuals, with over half of the world's population infected.¹ The prevalence of the infection is higher in the low socioeconomic and crowded populations.² *Helicobacter pylori* plays important role in the etiology of gastritis, gastric and duodenal ulcers³ but some studies are available showing *H. pylori* colonisation in adenoid and tonsil tissue.⁴⁻⁶ Aim : We assume there is colonization of *H. pylori* in chronic tonsillitis and determine the presence of *Helicobacter pylori* in chronic tonsillitis with H&E and Giemsa staining in the tonsillectomy specimens. Methods: This is a descriptive study with cross sectional which the sample will be obtained using consecutive sampling method. We collect 43 cases with chronic tonsillitis were examined in tonsillectomy specimens for which available tissue for routine staining could be retrieved were histopathology chronic tonsillitis. The colonization of the *Helicobacter pylori* have been evaluated with hematoxylin & Eosin (H&E) and Giemsa stains under the light microscope. Results: With H&E staining, *Helicobacter pylori* has been detected in 23 cases (53,48%) of the tonsillectomy specimens in total. No colonization has been observed in the remaining 20 cases (46,51%). With Giemsa staining *Helicobacter pylori* has been detected in 29 cases (67,74%) of the tonsillectomy specimens in total. No colonization has been observed in the remaining 14 cases (32,55%)

Keyword: *Helicobacter Pylori*, Chronic Tonsillitis, H&E, Giemsa

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1 Introduction

Helicobacter pylori (*H. pylori*) is a pathogen which is recognized as the most frequent infection of individuals. More than half of the world's population is being infected.¹ *H. pylori* plays an important role in the etiology of gastritis, gastric and duodenal ulcers.³ It spreads by oral-oral or faecal-oral way.⁷ According to these routes of transmission, there are reported studies on the colonization of *H. pylori* in upper respiratory tract and oral mucosa. Adenotonsillar tissue has been started to be considered as one of the reservoir for the microorganism.⁸⁻¹¹ The aim of this study is to determine the presence of *Helicobacter pylori* in cases of chronic tonsillitis in tonsillectomy specimens.

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2 Materials and methods

This is a descriptive study with cross sectional which the samples will be obtained using consecutive sampling method. We collect 43 paraffin block of the chronic tonsillitis cases from 2013-2017, were examined in tonsillectomy specimens for which available tissue for routine staining could be retrieved were histopathology chronic tonsillitis. Patients' age, gender were taken from the reports. The samples were taken from Laboratorium anatomical pathology medical faculty of Universitas of Sumatera Utara (USU), Instalation Anatomical pathology of RS Haji Medan, and RSUD Pirngadi Medan.

Discovered in 1983, *H. pylori* was first seen by Robin Warren using H&E staining. The use of giemsa staining or warthin-starry techniques has made it easier for anatomic pathologists to diagnose infections caused by *H. pylori*.

In this study, we use H&E and giemsa staining, because these is routine staining, cheaper, simple procedure, and giemsa showed sensitivity 85%, specificity 89% to *H.pylori*.¹² H&E procedure as below : deparaffinize the section, hydration with decreasing concentration of alcohol, stain in hematoxylin, wash in running tap, differentiate in 1% acid alcohol, wash in running tap, stain in 1% eosin Y, wash in tap water, dehydrate in increasing concentration of alcohols, clear in xylene, and mounting. Histochemical staining for giemsa was performed on the 3-4 μ m, formalin-fixed, paraffin-embedded sections to detect *H. pylori*. Giemsa staining was performed according to the standard procedure : First the slides were in air-dried smear, then stained with diluted Giemsa's staining solution for manual staining, brought buffer solution, then dehydrated rapidly, cleaned and mounted.

A case diagnosed as chronic gastritis with *H. pylori* colonization was used for the positive control. The presence of *H. pylori* was investigated on the mucosal surfaces in under the light microscope with both H&E and giemsa stained sections.

The colonization of the helicobacter pylori have been evaluated with hematoxylin & Eosin and Giemsa stains under the light microscope.

3 Results

This study was done in Department of Pathology, Faculty of Medicine, Universitas of Sumatera Utara. Histopathological investigation of the specimens confirmed the diagnosis of chronic nonspecific tonsillitis in 43 patients. The patients age range was between 9 - 64 years old of which 11 were males (25,6%) and 32 were females (74,4%). The mean age of the patients was 27,97 yr, and standard deviation \pm 15,4.

H. pylori was identified in the histopathological evaluation of H&E sections, 20 cases (46,51%) on one side of the bilateral tonsillectomy specimens while it has been seen in 3 cases (6,9%) on both sides which demonstrated positivity in 23 cases (48%) in total. No colonization has been observed in the remaining 20 cases (46,51%).

Table 1 Frequency Distribution of gender

Jenis kelamin	Jumlah (n)	Persentase (%)
Laki-laki	11	25,6
Perempuan	32	74,4
Jumlah	43	100,0

Table 2 The Presence of *Helicobacter pylori* in cases of Chronic Tonsillitis on H&E-stained sections

H&E staining	Number	Persentase (%)
Positif	23	53,48
Negatif	20	46,51
Total	43	100,00

The presence of *Helicobacter pylori* in tonsillectomy tissue with hematoxylin & eosin (H&E) staining was found to be positive in 20 cases (46.51%) on one side of bilateral tonsillectomy tissue and 3 cases (6.97%) were positive in both, and negative results in 20 cases (46.51%).

Table 3 The Presence of *Helicobacter pylori* in cases of Chronic Tonsillitis on Giemsa-stained sections

Giemsa staining	Number (n)	Persentase (%)
Positif	29	67,74
Negatif	14	32,55
Total	43	100,00

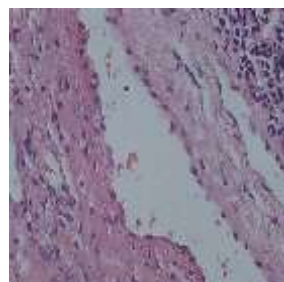
On giemsa-stained sections, *H. pylori* was detected in 26 cases (60,46%) on one side of the bilateral tonsil-lectomy specimens while it has been seen in 3 cases (6,9%) on both sides which demonstrated positivity in 29 cases (67,74%) in total. No colonization has been observed in the remaining 14 cases (32,55%).

4 Discussion

Chronic tonsillitis is a common upper respiratory tract disease mainly in children, caused by recurrent attacks of acute infections, pathogenic organisms, smoking, bad hygiene of mouth, whether, physical exhaustion, inadequate therapy, or when the child's immune system has decreased. The etiology of chronic tonsillitis is still unclear. Because it may affect the treatment methods, it is important to clarify the etiology.

H.pylori is a Gram-negative bacterium persistently found in gastric mucosa. It may be the most frequent chronic infection affecting humans, with over half of the world's population infected.¹ The prevalence of the infection is higher in the low socioeconomical and crowded populations⁹ especially in Indonesia . Some study describe the colonizations of *H.pylori* were to be present in the oropharyngeal area in subgingival plaques, dental plaques and saliva¹⁴, adenoid and tonsil tissue.⁴⁻⁶ Oral-oral, fecal-oral route is the most common route of transmission.⁷ Minocha et al. has reported a study about the importance of the colonization of *H. pylori* in tonsillary tissue. The decrease of the prevalence of gastric *H. pylori* has been pointed out in the cases with tonsillectomy.¹⁵

Figure 1 *H.pylori* like microorganism on the mucosal surface of the tonsillary tissue.



H&E staining, 400 X



Giemsa staining, 400 X

In this study, the tissue of chronic tonsillitis in females were 32 cases (74,4%) more than 11 cases (25,6%) in males (tabel 1), according to morbidity data by Survey Kesehatan Rumah Tangga (SKRT) 1995 reported that pattern of the disease in children were age range between 5-14, chronic tonsillitis was the 5th rank (10,5 % in boys, 13,7 % in girls).¹⁶ The results of Amalia N et al, they reported there was 67 cases (83,3%) of treatment and 13 cases (16,2%) of tonsillectomy.¹⁷

The patients age range was between 9 - 64 years old of which 11 were males. The mean age of the patients was 27,97 yr, and standard deviation $\pm 15,4$. we reported 20 cases (46,5%) of the age range between 17-25 and 2 cases of the age range between 5-11 (4,6%) with mean age

27,97 yr and standard deviation $\pm 15,4$. RSUP dr Kariadi Semarang reported the incidence of chronic tonsillitis was 23,36% which age range in 6-15 yr.¹⁸ Sapitri reported research on the characteristics of chronic tonsillitis sufferers indicated tonsillectomy in Raden Mattaher Hospital Jambi, from the 30 samples obtained the most distribution age 5-14 years (50%), female sex (56,7%) and have complaints of pain in the throat / swallowing pain (100%).¹⁹ Fakh IM et al., reported chronic tonsillitis patients in children are mostly found in the age group of 10-14 years as many as 50%, female sex as much as 56%, the main complaint of repeated swallowing pain as much as 56%, T3-T3 tonsil size as much as 68%, management operatively as much as 88%.²⁰ Based on the literatures, acute and chronic tonsillitis can be happen to all age, but incidence is often occurred in children. the main risk factors is from upper respiratory infection and inadequate treatment of acute tonsillitis.^{18,19,20} While the study of Amalia N et al reported that the common age of the chronic tonsillitis were age range between 36-47 y.o (26,3%).¹⁷

In this study, we found the presence of *H. pylori* in tonsillectomy tissue with hematoxylin & eosin (H&E) staining was positive in 20 cases (46,51%) on one side of bilateral tonsillectomy tissue and 3 cases (6,97%) were positive in both. But on giemsa-stained sections, *H. pylori* was detected in 26 cases (60,46%) on one side of the bilateral tonsil-lectomy specimens while it has been seen in 3 cases (6,9%) on both sides which demonstrated positivity in 29 cases (67,74%) in total.

H. pylori can be seen in routine H&E staining, but need good precision and high magnification are required and it is very difficult to see organisms in small numbers (non-colonies). H&E staining is much cheaper, but has the low-middle sensitivity.

Giemsa staining is inexpensive, easy to obtain and relatively simple technique. The difficulty in staining with Giemsa must be assessed with high magnification to be able to see the organism. The lack of this coloring will stain all bacteria and only display the classic morphology of *H. Pylori*.

There are many factors of pathogenesis in chronic tonsillitis, due to the etiology is still unclear. We assume there is colonization of *H. pylori* in chronic tonsillitis, by the presence of *helicobacter pylori* in chronic tonsillitis specimens with H&E and Giemsa staining. But in this study we only found 23 cases (53,48%) positive of *H. pylori* on H&E-stained sections and 29 cases (67,74%) positive on giemsa-stained sections from 43 samples in all.

5 Conclusion

There is a way to use H&E routine staining and Giemsa staining to detect *Helicobacter pylori*. According to this result, it is important to keep in mind the presence of *H. pylori* in chronic tonsillitis-tissue. Due to the complexity of the oral mucosa, other diagnostic tools are needed to confirm the diagnosis.

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